Physical Map Location of the *Rps1-k* Allele in Soybean

M. E. Gardner, T. Hymowitz, S. J. Xu, and G. L. Hartman*

ABSTRACT

Several genetic maps of soybean [*Glycine max* (L.) Merr.] have been developed during the past decade. Different markers have been used to construct these maps including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and classical markers. However, virtually none of the maps and linkage groups developed has been associated with specific soybean chromosomes. For example, there are 13 single dominant resistance genes at 7 different loci that control *Phytophthora sojae*. These resistance genes (*Rps* genes) have not been located on any chromosomes, but several have been associated on classical and molecular maps. For example, the *Rps1* locus is associated with molecular linkage group N. The objective of this study was to locate the *Rps1* locus on a specific soybean chromosome using primary trisomic analysis. Crosses were made between 10 soybean trisomic lines and cv. Resnik (containing *Rps1-k*). The F_2 populations from trisomic parents were inoculated with race 3 of *P. sojae* to determine the ratio of resistant to susceptible plants. Nine of the F_2 populations tested segregated in a normal 3:1 ratio. The F_2 population of triplo 3 segregated in a 2:1 ratio, the expected segregation ratio for a single dominant gene if the gene is located on the extra chromosome, suggesting that the *Rps1* locus is on chromosome 3. Thus, chromosome 3 corresponds to molecular linkage group N of the integrated genetic linkage map.

Phytophthora root rot of soybean, [*Glycine max* (L.) Merr.], is caused by *Phytophthora sojae* Kaufmann and Gerdemann. Phytophthora root rot is a problem in soybean growing regions in the United States and in other countries including Argentina, Australia, Brazil, Canada, and China. In 1994, Phytophthora root rot ranked third in the U.S. in yield-reducing diseases of soybean (Wrather et al., 1997). *Phytophthora sojae* induces a soft rot and browning of the root and lower stem, with an eventual collapse of tissues.

Genetic resistance is one of the most effective methods of controlling Phytophthora root rot. The major sources of resistance are a series of single dominant *Rps* genes (Schmitthenner, 1999). There are 13 *Rps* genes at seven different loci that provide race-specific resistance (Schmitthenner, 1999). Of these genes, five occur at the *Rps1* locus including *Rps1-k*, which was first identified from the cultivar Kingwa (Bernard and Cremeens, 1981). Since it was first identified, *Rps1-k* has become one of the most predominant resistance genes in modern soybean cultivars. Often the use of single dominant resistance genes creates selection pressure within the pathogen population. Ryley et al. (1998) found that changes in the race structure of the *P. sojae* population followed the deployment of specific resistance genes in Australia. Anderson and Buzzell (1992a) also believed that shifts in the race composition of *P. sojae* in Ontario could be partially explained by the host genotype deployed in the survey area. Leitz et al. (2000) reported new races in Illinois that are virulent on *Rps1-k*, which is a common source of resistance, and *Rps1-d*, which has not been widely used as a source of resistance. Since *P. sojae* was first described in 1948, more than 39 different races have been identified (Schmitthenner, 1999).

Various genetic maps of soybean have been developed by using different markers like SSRs, RFLPs, RAPDs, AFLPs, and classical markers to construct these maps (Akkaya et al., 1995; Cregan et al., 1999; Kasuga et al., 1997; Keim et al., 1997; Lark et al., 1993; Shoemaker and Specht, 1995). Twenty classical linkage groups have been identified that contain morphological, pigmentation, and disease resistance genes (Cregan et al., 1999). Several *Rps* genes have been located on classical genetic linkage groups in soybean. The *Rps2* gene was shown to be linked with *Rmd*, which confers resistance to *Microsphaera diffusa*, and *Rj2* the nodulation response gene in linkage group J (Devine et al., 1991; Lohnes et al., 1993; Polzin et al., 1994). The *Rps1* locus has been linked to the genes for pod wall color (Kilen, 1979) and metribuzin insensitivity (Kilen and Barrentine, 1983) as well as *Rps7* (Anderson and Buzzell, 1992b). The linkages involving *Rps1* form linkage group 10. The *Rps1* locus also has been identified to various molecular maps.

Using RFLP markers, Diers et al. (1992) determined that *Rps1-k* is on linkage group K (Keim et al., 1990). This linkage group (LG) was later designated LG-N by Shoemaker and Olsen (1993). Lohnes and Schmitthenner (1997) were able to associate LG-22 from the Clark × Harosoy isoline map and LG-10 of the classical map with LG-N of the public RFLP map. Kasuga et al. (1997) used RAPD and AFLP analyses to create a genetic map of the region containing *Rps1-k*. Classical linkage group 10 containing *Rps1-k* has been associated with linkage group N-U06 from the University of Utah (Minsoy × Noir 1) map, linkage group N-ISU from the USDA/Iowa State University (*G. max* × *G. soja*) map and linkage group N-CH22 from the University of Nebraska (Clark × Harosoy) map. In soybean, the genetic linkage map as well as the molecular based maps have not been associated with specific soybean chromosomes. In other major crop plants these classical and molecular maps have been superimposed on their respective chromosome maps.

One method of creating a soybean genome map is by determining the chromosomal location of genes, such

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Abbreviations: RFLP, restriction fragment length polymorphism; AFLP, amplified fragment length polymorphism; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeat.

Table 1. Segregation ratios and Chi-square values of trisomic F_2 soybean populations segregating for *Rps1-k*.

Trisomic†	Total no. of plants	No. of plants alive	No. of plants dead	Theoretical ratio	Chi-square Value	Chi-square Prob.
Triplo 1*	257	189	68	3:1	0.29	0.59
Triplo 2*	372	272	100	3:1	0.7	0.40
Triplo 3	217	136	81	3:1	17.59	<0.01
Triplo 3	217	136	81	2:1	1.56	0.21
Triplo 4	58	47	11	3:1	1.13	0.29
Triplo 5*	468	347	121	3:1	0.18	0.67
Triplo 10	149	106	43	3:1	1.18	0.28
Triplo 15	241	175	66	3:1	0.73	0.39
Triplo 16*	233	181	52	3:1	0.89	0.34
Triplo 17	284	208	76	3:1	0.47	0.49
Triplo 20	104	85	19	3:1	2.51	0.11

† Asterisk (*) indicates combined analysis of seeds harvested from two plants that show no heterogeneity.

as *Rps1-k*, that are found in both classical linkage and molecular maps. Determining the chromosomal location of genes can be accomplished through primary trisomic analysis. A primary trisomic has one additional complete chromosome, altering its chromosome number from $2n = 2x$ to $2n = 2x + 1$. Such an individual is called a triplo (Singh, 1993). Primary trisomics can be used to locate a gene on a particular chromosome, verify the independence of linkage groups, and associate linkage groups with individual chromosomes. This method has been used to determine gene-chromosome relationships in several plant species (Singh, 1993). The chromosomal location of a specific locus can be determined by the altered segregation ratios in the F_2 offspring of trisomics. In these offspring, genetic ratios will be modified from the expected 3:1 F_2 ratio for a dominant gene. The new ratio is dependent on the genotypes of the F_1 primary trisomic plants, on the type of chromosome segregation, and on the female transmission rate of the extra chromosome (Singh, 1993). The average female tansmission rate of the 20 primary soybean trisomics was 42% with a range of 27 (triplo 20) to 59% (triplo 9) (Xu et al., 2000). The pollen transmission of the extra chromosome is very low due to the inability of pollen with $(n + 1)$ chromosomes to compete with pollen with n chromosomes.

An integrated soybean genome map would be beneficial to scientists and breeders. Such a map would have applications in functional genomics and marker assisted breeding. Identifying the placement of the *Rps1-k* gene on the soybean physical map will create a more complete view of the soybean genome by also associating molecular and classical linkage groups that contain the *Rps1-k* locus. The objective of this study was to locate the *Rps1* locus on a specific soybean chromosome using primary trisomic analysis.

MATERIALS AND METHODS

The complete set of twenty primary trisomics for soybean $(2n = 40 + 1)$ were provided by T. Hymowitz, Dep. of Crop Sciences, University of Illinois. Primary trisomics had been identified from diverse backgrounds using pachytene chromosome analysis and backcrossed into cv. Clark 63 background (Ahmad and Hymowitz, 1993; Ahmad et al., 1992; Singh and Hymowitz, 1990; Xu et al., 1998a, 2000). The trisomic lines were first inoculated with race 3 of *P. sojae* to determine their reaction and verify their susceptibility to this race. Cultivar Clark 63 (contains *Rps1-a*), which is the recurrent parent for the trisomic lines, was used as the susceptible check and cv. Resnik (contains *Rps1-k*) as the resistant check. Race 3 of *P. sojae* was used because it is virulent on *Rps1-a* and avirulent on *Rps1-k*. Soybean seeds were planted in 10-cm-deep flats containing a 1:1 mix of steam-pasteurized soil and sand. Nine entries were planted in each tray with five seeds per entry. Seedlings were grown for 10 to 12 d in a greenhouse with a photoperiod of 16 h and watered daily.

Seedlings were inoculated by the hypocotyl plug method (Pazdernik et al., 1997). *Phytophthora sojae* isolates were grown on lima bean agar for 10 to 12 d. A cork borer was used to cut 3-mm-diameter plugs along the leading edge of the colonies. Plugs were placed mycelial side down on the cotyledon, touching the stem. One *P. sojae* culture was used per tray. The trays and the inside of the clear plastic domes were misted with water from an atomizer. The domed trays were placed under black shade cloth (80% light reduction) for 4 d. The environment during the incubation period averaged 25°C and 95% humidity. At the end of 4 d domes were removed. Trays remained under the shade cloth for an additional 2 d. Reactions of each entry were recorded 6 d after inoculation. Resistance was determined on the basis of percentage survival per entry.

Trisomic plants used for crossing were first identified by determining their chromosome number from root tip samples. The procedure used is as described by Xu et al. (1998b). Five seeds from each line were grown in vermiculite for 5 to 7 d. Seedlings were harvested, 1-cm samples were taken from young roots and collected in 1.5 mL microcentrifuge tubes containing double distilled water. After the samples were taken, seedlings were replaced in the vermiculite.

The water was removed from the tubes and root tips were pretreated in 0.05% 8-hydroxyquinoline for 5 h at 16°C in a Micro Cooler to arrest the chromosome metaphase cells. Root tips were then fixed in a 3:1 mixture of 95% ethanol and propionic acid and left overnight at room temperature. The fixative was removed from the tubes and samples were then washed twice with double distilled water. Root tips were hydrolyzed in 1N HCL at 60°C for 16 min. To stain the chromosomes, root tips were first washed once with double distilled water and then placed in Feulgen stain at room temperature for 2 h. The Feulgen stain was then removed and root tips were rinsed with cold double distilled water (1 to 4°C). After two rinses, Carbol staining solution was added to the samples. Root tips were left in this solution overnight at 1 to 4°C. After 14 to 16 h, the staining solution was removed and the root tips were washed 3 to 4 times with cold double distilled water to remove any residual stain. Root tips were then stored in cold water in the refrigerator until needed. To prepare slides, root tips were squashed in a drop of 45% acetic acid.

Seedlings that were identified as having 41 chromosomes were then transplanted to clay pots in a 1:1:1 mixture of sand, soil, and perlite. Plants were grown in the greenhouse and crosses were made between the trisomic female (rrr) and cv. Resnik male (Rr). F_1 seeds were harvested from crosses and planted in vermiculite. Chromosome numbers of the F_1 plants were determined in the same manner as described above. Plants identified having 41 chromosomes (Rrr) were grown in the greenhouse and allowed to self-pollinate.

F_2 seeds were harvested and planted in 10-cm-deep flats containing a 1:1 mix of steam-pasturized soil and sand. All of the trays contained five seeds each of cv. Clark 63 and cv. Resnik for use as a susceptible check and a resistant check, respectively. In each tray approximately 45 F_2 seeds from the trisomic × cv. Resnik crosses were planted. All seeds from each individual cross were kept separate and planted in separate trays. The inoculation method used was the same as described above. A chi square goodness-of-fit test was performed on each F_2 population with the Statistical Analysis System (SAS Institute, 1992).

RESULTS AND DISCUSSION

All twenty trisomic lines were susceptible when inoculated with race 3 of *P. sojae*. Crosses were made with 10 of the possible 20 trisomic lines. At the onset of this project no information was available to indicate the chromosome location of *Rps1-k*, thus half of the trisomic lines were chosen at random to cross. Crosses were made with triplos 1, 2, 3, 4, 5, 10, 15, 16, 17, and 20.

The average survival of cv. Resnik (resistant check) was 94% and the average survival of cv. Clark 63 (susceptible check) was 5%. The F_2 populations from triplos 1, 2, 4, 5, 10, 15, 16, 17, and 20 fit the expected 3:1 segregation ratio for a single dominant gene (Table 1). The F_2 population from triplo 3 did not fit this ratio. This population segregated in a 2:1 ratio, which is based on the 50% female and 0% male transmission rate of an extra chromosome from the F_1's with simplex (Aaa) genotype.

To determine the difference between a 3:1 and a 2:1 segregation ratio, ideally 400 to 500 seed would be used. In this study, the numbers of seed tested ranged from 58 to 468. While only 217 seeds of triplo 3 were tested, the segregation ratio obtained of 136 resistant: 81 susceptible, did not agree with the expected 3:1 ratio, but did agree with a 2:1 ratio. According to these results, the *Rps1-k* gene is located on chromosome 3. Since the *Rps1* locus has been reported on molecular linkage group N, then molecular linkage group N and also *Rps7* must be located on chromosome 3.

At this time there are 20 defined classical linkage groups containing 68 classical genes (Cregan et al., 1999). All but one of these linkage groups has been associated with a molecular linkage group (Cregan et al., 1999). Most of the known genes in soybean have not been identified to specific chromosomes (Xu et al., 1998a). Almost none of the genetic linkage groups and molecular maps have been associated with specific chromosomes (Xu et al., 1998a).

Xu et al. (2000) associated two seed protein genes *eu1* and *lx1* and a morphological marker gene *p2* to chromosomes 5, 13, and 20, respectively. By using primary trisomic analysis, it is possible to associate genes and linkage groups to specific chromosomes and thereby very quickly create a universal soybean genome map.

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REFERENCES

Ahmad, F., and T. Hymowitz. 1993. Identification of five new primary trisomics in soybean based on pachytene chromosome analysis. Genome. 37:133–136.

Ahmad, F., R.J. Singh, and T. Hymowitz. 1992. Cytological evidence for four new primary trisomics in soybean. J. Hered. 83:221–224.

Akkaya, M.S., R.C. Shoemaker, J.E. Specht, A.A. Bhagwat, and P.B. Cregan. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci. 35:1439–1445.

Anderson, T.R., and R.I. Buzzell. 1992a. Diversity and frequency of races of *Phytophthora megasperma* f. sp. *glycinea* in soybean fields in Essex County, Ontario, 1980–1989. Plant Dis. 76:587–589.

Anderson, T.R., and R.I. Buzzell. 1992b. Inheritance and linkage of the *Rps7* gene for resistance to Phytophthora rot of soybean. Plant Dis. 76:958–959.

Bernard, R.L., and C.R. Cremeens. 1981. An allele at the *Rps1* locus from the variety Kingwa. Soybean Genet. Newsl. 8:40–42.

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. VanToai, D.G. Lohnes, J. Chung, and J.E. Specht. 1999. An integrated genetic linkage map of the soybean genome. Crop Sci. 39:1464–1490.

Devine, T.E., T.C. Kilen, and J.J. O'Neill. 1991. Genetic linkage of the Phytophthora resistance gene *Rps2* and the nodulation response gene *Rj2* in soybean. Crop Sci. 31:713–715.

Diers, B.W., L. Mansur, J. Imsande, and R.C. Shoemaker. 1992. Mapping Phytophthora resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci. 32:377–383.

Kasuga, T., S.S. Salimath, J. Shi, M. Gijzen, R.I. Buzzell, and M.K. Battacharyya. 1997. High resolution genetic mapping and physical mapping of the molecular markers linked to the Phytophthora resistance gene *Rps1-k* in soybean. Mol. Plant. Microb. Inter. 10:1035–1044.

Keim, P., B.W. Diers, T.C. Olson, and R.C. Shoemaker. 1990. RFLP mapping in soybean: Association between marker loci and variation in quantitative traits. Genetics 126:735–742.

Keim, P., J.M. Schupp, S.E. Travis, K. Clayton, T. Zhu, L. Shi, A. Ferreira, and D.M. Webb. 1997. A high-density soybean genetic map based on AFLP markers. Crop Sci. 37:537–543.

Kilen, T.C. 1979. Observations on possible linkage between the *Rps1* locus and pod wall color. Soybean Genet. Newsl. 19:156–158.

Kilen, T.C., and W.L. Barrentine.1983. Linkage relationships in soybean between genes controlling reactions to Phytophthora rot and metribuzin. Crop Sci. 23:894–896.

Lark, K.G., J.M. Weisemann, B.F. Matthews, R. Palmer, K. Chase, and T. Macalma. 1993. A genetic map of soybean (*Glycine max* L.) using an intraspecific cross of two cultivars: 'Minsoy' and 'Noir 1'. Theor. Appl. Genet. 86:901–906.

Leitz, R., G.L. Hartman, W.L. Pedersen, and C.D. Nickell. 2000. Races of *Phytophthora sojae* on soybean in Illinois. Plant Dis. 84:487.

Lohnes, D.G., and A.F. Schmitthenner. 1997. Position of the Phytophthora resistance gene *Rps7* on the soybean molecular map. Crop Sci. 37:555–556.

Lohnes, D.G., R.E. Wagner, and R.L. Bernard. 1993. Soybean genes *Rj2*, *Rmd*, and *Rps2* in linkage group 19. J. Hered. 84:109–111.

Pazdernik, D.L., G.L. Hartman, Y.H. Huang, and T. Hymowitz. 1997. A greenhouse technique for assessing Phytophthora root rot resistance in *Glycine max* and *G. soja*. Plant Dis. 81:1112–1114.

Polzin, K.M., D.G. Lohnes, C.D. Nickell, and R.C. Shoemaker. 1994. Integration of *Rps2*, *Rmd*, and *Rj2* into linkage group J of the soybean molecular map. J. Hered. 85:300–303.

Ryley, M.J., N.R. Obst, J.A.G. Irwin, and A. Drenth. 1998. Changes in the racial composition of *Phytophthora soja* in Australia between 1979 and 1996. Plant Dis. 82:1048–1054.

SAS Institute. 1992. SAS/STAT user's guide. Version 6. 4th ed. SAS Inst., Cary, NC.

Schmitthenner, A.F. 1999. Phytophthora root rot. *In* G.L. Hartman, J.B. Sinclair, and J.C. Rupe (ed.) Compendium of Soybean Diseases. 4th ed. APS Press. Am. Phytopath. Soc., St. Paul, MN. p. 39–42.

Singh, R.J. 1993. Plant Cytogenetics. CRC Press Inc., Boca Raton, FL. p. 131–159.

Singh, R.J., and T. Hymowitz. 1990. Identification of four primary trisomics of soybean by pachytene chromosome analysis. J. Hered. 82:75–77.

Shoemaker, R.C., and T.C. Olson. 1993. Molecular linkage map of soybean (*Glycine max* L. Merr). p. 6.131–6.138. *In* S.J. O'Brien (ed.) Genetic maps: Locus maps of complex genomes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Shoemaker, R.C., and J.E. Specht. 1995. Integration of the soybean molecular and classical genetic linkage groups. Crop Sci. 35:436–446.

Wrather, J.A., T.R. Anderson, D.M. Arsyad, J. Gai, L.D. Ploper, A. Porta-Puglia, H.H. Ram, and J.T. Yorionori. 1997. Soybean disease loss estimates for the top 10 soybean producing countries in 1994. Plant Dis. 81:107–110.

Xu, S.J., R.J. Singh, and T. Hymowitz. 1998a. Establishment of a cytogenetic map of soybean: Current status. Soybean Genet. Newsl. 25:120–122.

Xu, S.J., R.J. Singh, and T. Hymowitz. 1998b. A modified procedure for mitotic chromosome count in soybean. Soybean Genet. Newsl. 25:107.

Xu, S.J., R.J. Singh, K.P. Kollipara, and T. Hymowitz. 2000. Primary trisomics in soybean: Origin, identification, breeding behavior, and use in gene mapping. Crop Sci. 40:1543–1551.

Genetic Analysis of Seedling Growth under Cold Temperature Stress in Grain Sorghum

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ABSTRACT

Planting of grain sorghum [*Sorghum bicolor* (L.) Moench] earlier in the season should contribute to a longer growing season and more effective utilization of late spring and early summer rainfall, but early planting is hindered by poor seedling vigor under cold temperature conditions. Sources of cold tolerance have been identified, but little is known about the inheritance of this trait. This study was conducted to determine combining ability for seedling growth characteristics under cold temperature conditions in genetically diverse sorghum lines using a Design-II mating scheme. Parental lines and hybrids were evaluated under early and normal planting conditions in the field. The traits measured included emergence, emergence index, leaf number, vigor, and dry weight. Significant differences were observed among entries. Among hybrids, these differences were primarily due to effects of general combining ability. These results indicate that general combining ability is more important than specific combining ability in breeding for seedling vigor in sorghum. Heterosis had favorable effects on all seedling traits measured.

In recent years, farmers and agronomists have emphasized earlier planting dates for grain sorghum. Early planting should contribute to a longer growing season, more effective utilization of late spring and early summer rainfall, and enhanced yield potential. Sorghum originated in the semi-arid tropics and expresses excellent heat and drought stress tolerance, but generally is susceptible to cold stress and often expresses poor early-season vigor. Therefore, sorghum germplasm with improved cold tolerance will need to be developed to harness these potential benefits of early planting.

Crop production also has shifted from conventional tillage to minimum tillage and no-till strategies. These conditions generally result in a decreased soil temperature at planting, because the crop residue insulates the soil and reflects solar radiation (Wall and Stobbe, 1983). Consequently, cultivars with vigorous early growth also are required to maximize the potential of early planting in these environments.

Sources of cold tolerance have been identified in sorghum (Soujeole and Miller, 1984; Nordquist, 1971; Singh, 1985). Genetic variability for cold tolerance is expressed as variations in germination, emergence, and seedling vigor under early planting conditions. However, little is known about the expression or inheritance of these traits in sorghum. The objective of this study was to evaluate combining ability for seedling growth characteristics under cold temperature conditions in genetically diverse sorghum lines.

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MATERIALS AND METHODS

The hybrids used in this study were developed by intercrossing four common sorghum seed-parent lines, 'Wheatland', SA3042, 'Redlan', and TxARG-1, with five diverse pollinator lines, Tx2737, Tx435, P954063, IS4225, and 'ShanQuiRed' (SQR), using a Design II mating scheme (Comstock and Robinson, 1952). Seed for all entries was produced in a winter nursery in Puerto Rico.

Parent lines and F1 hybrids were evaluated for seedling growth traits under early and normal planting conditions at experiment field sites in Manhattan, KS, on a Eudora-Muir soil type and Hesston, KS, on a Ladysmith-Goessel soil type in 1999 and 2000 (Table 1). Each experiment was conducted using a randomized complete block design with four replications. Entries were grown in single-row plots (5.08m $\times$ 0.76m), and 75 seeds were planted per row. Seeds for each entry were treated with N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (Captan) fungicide at the label rate.

Seedling dry weight (above ground), leaf number, emergence, emergence index, and vigor score were measured to evaluate differences in seedling growth. Stand counts were recorded every other day after planting until no more seedlings emerged. Emergence index, a measurement of rate of emergence, was calculated as described by Smith and Millett (1964) using the formula:

$$\text{Emergence Index} = \Sigma\ (E_j \times D_j)/E$$

where E_j = emergence on day j, D_j = days after planting, and E = final stand. Final stand counts were taken at 30 d after planting. Ten random plants were harvested (above ground only) from each plot at 30 d after planting and were used to measure the number of fully expanded leaves and to calculate seedling dry weight. Twenty-five days after planting, vigor scores from 1 to 5 (1 = excellent; 5 = poor) were assigned to each plot as described by Maiti (1996).

Analyses of variance and combining ability were carried out per established methods (Hallauer and Miranda, 1988; McIntosh, 1983) using the PROC GLM procedure of the SAS Statistics package (SAS, 1989). Several traits required transformation to improve the homogeneity of the error variance. Data for emergence were transformed by arcsine transformation (Little and Hills, 1978). Data for seedling dry weight were transformed by $\log_{10}$ (mg) logarithmic transformation (Menkir